Amendment to the Specification

In the Specification:

In accordance with the provisions of 37 C.F.R. § 1.121(b), please amend the claims as follows:

At pages 16-17, please replace the paragraph starting under the heading **Crystallinity** with:

E1

-- Generally, careful control of the adjuvant's degree of crystallinity and crystal size may be used to affect the overall vehicle resorption rate. For apatitic calcium phosphates with calcium to phosphorous ratios of 1.3-1.75, poorly crystalline forms are believed to resorb more quickly than highly crystalline forms. Highly crystalline ~~stoichimetric~~ stoichiometric hydroxyapatite (e.g., NIST® catalog # 2910) is an example of a weakly resorbable vehicle. For other calcium phosphates, for a given calcium to phosphorous ratio, more amorphous forms will generally be more soluble than more crystalline forms. Increased resorption rates may be achieved through the production of apatitic calcium phosphates containing lattice defects, such as ionic vacancies or substitutions. Preferred embodiments include carbonated or otherwise calcium deficient apatites, all of which tend to have increased *in vivo* resorption rates. Further guidance for the production of similar such apatic calcium phosphates can be found in Structure and Chemistry of the Apatites and Other Calcium Orthophosphates, (Elsevier, Amsterdam, 1994, by J.C. Elliott), and the references contained therein, all incorporated herein by reference. --

At pages 26-27, please replace the paragraph starting under the heading **Selective immune response enhancement** with:

E2

-- A variety of enhancement means, both endogenous and exogenous may be used to augment, enhance or, in some instances, suppress specific components of the immune response. Macrophages (e.g. Mac-3), T cells (e.g. ~~CD3e~~ CD3ε, CD4, ~~CD8a~~ CD8α) and B cells (e.g. CD45R/B220) may be selectively augmented after injection or implantation of different calcium phosphate adjuvants (Example 18), which by virtue of their specific formulation, have endogenously enhanced adjuvanticity. Manipulation of specific immune response components can also be affected through a choice of: administration routes, adjuvant combinations, antigen

~~useage~~ usage, immunizing in the presence of cytokines, and/or using various carriers. To apply these approaches of adjuvant manipulation for a particular vaccine, it is helpful to identify which component of the immune system to target (e.g. humoral or cellular). Eventually, all components of the immune system function to protect the body from an attack, but in some cases, it is desirable to first elicit one component over the other. For example, eosinophils are desired to protect against parasitic infections and mast cells are important in allergic responses. Additionally, the T-helper cell types may be elicited when a cell-mediated immune response is desired and B-cells may be elicited when a humoral response is desired. Additionally, CD8 cells may be recruited or cytokines may be incorporated with the adjuvant when targeting tumor tissue since these cell types are known to combat tumor tissue. Once the desired type of cell response is identified, the above parameters may be adjusted as described herein, to elicit an enhanced response from the desired cell or cell types. Table 1 in Example 18 provides guidance for selecting an appropriate adjuvant formulation, based on the desired immune response. The level of cellular immune response is indicative of the adjuvant activity of the prepared calcium phosphate adjuvant, the higher the response, the greater the adjuvant activity. For example, a specific ACP adjuvant (prepared according to example 7) is used to elicit an augmented B-cell and macrophage response. Further guidance can be found in Golding (~~Annuals~~ Annals New York Academy of Sciences, 754, 1995, p127-137) and Newman (The Journal of Immunology, 148, 1992, p2357-2362). --

E2 Cont'd

At pages 34-35, please replace the paragraph under the heading **Manufacturing the Adjuvant** with:

E3

-- The calcium adjuvants of the present invention can be manufactured to resorb and/or deliver immunogens according to the desired need. Parameters that may be adjusted according to need include: surface treatments, porosity, particle size, and others. Calcium phosphates, such as hydroxyapatites, prepared under low temperature conditions and PCA calcium phosphates are preferred adjuvants, along with amorphous calcium phosphates. The preparation of these calcium phosphates is described respectively in co-pending U.S.S.N. 08/554,817 and issued

patents 5,650,176 by Lee ~~et.~~et al., 5,676,976 by Lee et al., and 5,683,461 by Lee ~~et.~~et al., and examples 1-17 herein. In preferred embodiments, the adjuvants are prepared as a gel, paste, pellets, gauge/mesh or sintered blocks. Gels are generally formed by precipitation. The precipitate aging time is varied to produce different particle sizes. Generally, the longer the aging time the larger the particle size. In some embodiments, after filtration and washing, the gel can be used as the adjuvant material. In other embodiments, the gel is mixed with an aqueous medium, such as physiological/sterile saline or water, at various concentrations to make a paste or putty. In yet other embodiments, the gel is ~~lyopholized~~ lyophilized to form a powder. The powder is mixed with an aqueous medium, such as saline, at different concentrations to form a paste or putty. The paste or gel can be allowed to harden. In preferred embodiments, an apatitic calcium phosphate adjuvant will harden at about 37°C, or body temperature. The hardened material can be broken, ground, milled or crushed to form particles of various sizes. The particles sizes can then be modified or adjusted as discussed above. These calcium compounds can be configured as adjuvants by combining active agents by any suitable means known in the art (e.g. through adsorption, co-precipitation or through the use of binders or fillers). --

At page 36, please replace the first paragraph with:

-- Alternatively, the gel can be ~~lyopholized~~ lyophilized to form a dry powder. The powder's (either lyophilized or non-lyophilized) particle size can be controlled by sieving, milling and treating with different temperatures. Sieving the powder allows the particles to be separated according to size, usually from smallest to largest. In preferred embodiments, the powder is milled. In most cases, a longer milling time results in a smaller particle size. In other embodiments, the powder is heated to different temperatures. Higher temperatures (e.g. 400°C-600°C) can be employed to increase the crystallinity of ACP powders. However, for temperatures up to about 450°C, the amorphous character of an ACP is preserved, but the specific surface area decreases, which marks a decrease in particle size. In other embodiments, a hardened form of the calcium-based adjuvant may be broken down into particles by milling, pulverizing and other methods. Fine powders of the nanometer or less size range (e.g. ACP), can

E4 cont'd

be compressed in a mold (e.g. compressive strength: 500 psi), then milled and sieved to achieve desired particle sizes. Other methods known in the art used to control particle size are considered to be within the scope of the invention. --

At page 38, please replace the first complete paragraph with:

E5

-- In other embodiments, the active agent is entrapped in the inventive calcium-based adjuvant. The active agent may ~~agglomerated~~ agglomerate with the adjuvant particles, to form a particle/agent agglomerate. In yet other preferred embodiments, the active agent is otherwise attached to the calcium adjuvant. The attaching methods include, but are not limited to, dipping, rolling, spraying, pressing, gluing, pasting, and painting. Alternatively, the active agent and/or other moieties may be present in fillers or binders used in device fabrication. These methods and others are well known in the art. Chemical bonding (covalent, ionic, and hydrogen) is another method used to combine the adjuvant and the antigen. In one preferred embodiment, the immunogen is covalently attached, by methods known in the art, to the vehicle through the use of a linker which is sensitive to proteolysis. --

At page 41, please replace the second paragraph with:

E6

-- The inventive calcium adjuvant may be chosen independently of the active agent to be delivered to a host. In other cases, tests can be performed in order to determine an optimal antigen-adjuvant match. The adjuvant's binding capacity with the antigen in the presence of various ionic conditions and buffering agents may be screened (e.g. fluorescent labeling or radioactive labeling) and the best match is chosen. ~~methods~~ Methods for assessing binding of moieties to solid calcium phosphate in varying ionic strengths are known to the art. Efficient incorporation of the antigen or other moiety occurs when the antigen interacts strongly with the base molecule and becomes bound to it. In these cases, the antigen is most likely to remain bound to the molecule and become entrapped in the adjuvant. --

Please replace the last paragraph on page 52 and continuing on page 53 with:

E7

-- On completion of the necropsy, the animal identification marks were retained (but not processed) in 10% neutral buffered formalin. For terminally euthanized animals, brain, spleen, thymus, right and left dorsal thoracic/scapular skin (injection sites), mandibular lymph node, liver and lung were trimmed and preserved. For each animal of the study, the right subcutaneous injection site was prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin. The left injection site was evaluated with the following antibodies: CD3, CD4, CD8, CD45R/B220 (or alternatively CD19) and Mac-3. The right injection sites of terminal mice were directly embedded in OCT medium, frozen and stained with ~~CD3e~~ CD3ε, CD4 and ~~CD8a~~ CD8α. Left injection site of terminal mice were immediately fixed with 10% neutral buffered formalin for 6 to 24 hours followed by embedding in paraffin wax and staining with hematoxylin and eosin and CD45R/B220 and Mac-3. Tissue was cut to 6 ~~um~~ μm sections with a cryostat for frozen tissues or rotary microtome for paraffin-embedded tissues, and stained for 60 minutes with Pharmingen monoclonal rat or hamster anti-mouse primary antibodies against Mac-3 or several CD antigens (~~CD3e~~ CD3ε, CD4, ~~CD8a~~ CD8α and CD45R/B220). --

At page 53, please replace the first complete paragraph with:

E8

-- A labeled streptavidin-biotin complex/HRP detection system (Dako No. K377) with an AEC chromogen was used to visualize the antigens. Sections were counterstained with Gill's III hematoxylin. Anti-hamster IgG (for ~~CD3e~~ CD3ε), goat anti-rat Ig (for ~~CD8a~~ CD8α, CD45R/B220 and CD4) and mouse anti-rat IgG1/IgG2a (for Mac-3) and hamster IgG isotype standard (for ~~CD3e~~ CD3ε), were appropriately diluted and substituted as the primary antibody on negative reagent control tissue sections in order to verify the specificity of the reaction. --

At page 55, in Table 1, please replace - - CD8a - - with - - CD8α - -

At page 57, please replace the first complete paragraph with:

E9

-- A time-related increase incidence of the groups with positive T lymphocytes (~~CD3e~~ CD3ε, CD4 and ~~CD8a~~ CD8α) was observed. After each euthanasia period, CD4 was the most common type of positive T cell observed in the various adjuvants. At 7 and 14 days post treatment, ~~CD3e~~ CD3ε was the second most commonly observed T cell. These results are reported in the following Table 2: --

At page 57, in Table 2 please replace - -CD3e- - with - - CD3ε - - and - - CD8a -- with --CD8α - -

At page 57, please replace the second paragraph with:

E10

-- In these groups, the amount of T cells were usually graded as slight (1-3 cell/field/40X) for mice euthanized at 72 hours and 7 and 14 days post treatment. The immunohistochemical detection of these different types of T cells at the injection site. Although the number of T lymphocytes was usually lower than the number of B cells and macrophages, their presence at the injection site indicate an active local immune reaction in response to the adjuvant. An absence or lower quantity of T lymphocytes was noted in mice treated with adjuvant prepared according to ~~examples 4,5,7,8,~~ examples 4, 5, 7, 8, and 13 compared with those treated with the other adjuvants. Examples 12 and 13 produced a delayed T cell reaction to CD3, while a transient T cell reaction to CD3 was observed in example 6. ~~Examples11~~ Examples 11 and 12 produced a delayed T cell reaction to CD4. Example 4 produced a transient T cell reaction to ~~CD8a~~ CD8α, while example 12 produced a delayed T cell reaction to ~~CD8a~~ CD8α. --

At page 58, please replace the first complete paragraph with:

E11

-- In preferred embodiments, a cytokine will be combined with the adjuvant according to its action to further increase the adjuvant effect. For example, cytokines, such IFN-γ, IL-1α and IL-2β will be combined with the selective adjuvants shown to induce macrophage responses. In other cases, an adjuvant prepared according to examples 1, 3 or 5 would be combined with IL-4 or IL-13 to increase the B cell response. Additionally, such cytokines as CD27 ligand, IL-2, and

E11 cont'd

IL-8 would be added to the adjuvants of examples 4, 6, 11, 12 and 15 to produce ~~enhance~~ enhanced T cell reaction. Further guidance for cytokine selection can be found in Appendix II: Cytokines and their Receptors (ImmunoBiology: The Immune System in Health and Disease, Janeway-Travers, Current Biology Ltd./Garland Publishing Inc., 1996), herein incorporated by reference. --

At page 59, please replace the last pragraph continuing onto page 60 with:

E12

-- All solutions were prepared sterilized. The calcium adjuvant is prepared according to examples 1-16, with the following modifications. The *Bordetella pertussis* (commercially available from Pasteur Vaccins) bacilli; killed and centrifuged, are homogenized in a 0.07 M dibasic sodium phosphate sterile solution so as to obtain $4x10^{10}$ bacilli per ml. The bacterial suspension of germs thus obtained is mixed with Solution A prior to mixing with Solution B. *B. pertussis* becomes absorbed to the calcium phosphate precipitate of the present invention.--

At page 66, please replace the first paragraph with:

E13

-- The calcium adjuvant was prepared according to ~~example 1and~~ example 1 and example 17. Commercially prepared hepatitis B vaccine (Hevac B) and diphtheria-tetanus-pertussis (DTP)-polio vaccine are available from Pasteur Vaccins. The vaccines are inactivated with formaldehyde and for the adjuvant of example 1 are combined with the adjuvant during the mixing of Solutions A and B. For the adjuvant of example 17 the vaccine is included in the hydration media at a concentration of 5.0 mg/ml. The paste is subsequently hardened at 37°C in a humid environment, then pulverized to a particle size of 250 nm. The prepared hydrated adjuvant is injected subcutaneously. A second injection is given 6 months later and a third injection again in 6 months. Blood samples are taken the day of the first injection and each subsequent injection. HB virus seric markers (HbsAg, anti-HBs, anti-HBc) are tested for with commercial radioimmunoassays (Abbott Laboratories). Anti-toxin titers to tetanus and diphtheria are determined by the passive hemagglutination techniques using highly purified

E 13 cont'd

toxins that are coupled to turkey erythrocytes by glutaraldehyde. For determination of pertussis agglutinins, measurements are made using the agglutination test performed in microtiter plates. --